SERUM LIPOCALIN-2 PREDICTS CARDIOVASCULAR MORBIDITY RISKS IN SUBCLINICAL HYPOTHYROID RAT MODEL

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ABSTRACT

Background: Subclinical hypothyroidism (SCH) and its associations with cardiovascular disease (CVD) remain controversial. Previous studies revealed that lipocalin -2 (LCN2) associated with increased cardiovascular mortality risk.

Objective: Evaluation of the serum level of LCN2 and its association with some cardiovascular risk factors in SCH rat model.

Material and methods: Twenty four adult male albino rats weighing 150-170 g were used and divided into two equal groups: control euthyroid group and SCH-induced group where SCH was induced by administration of methimazole (MMI) (10 mg/kg body weight) by gavage once daily for 3 months. Groups were compared for body mass index (BMI), serum thyroid-stimulating hormone (TSH), triiodothyronine (T3) thyroxin (T4), glucose, insulin, lipid profiles, C reactive protein (CRP), LCN2, Homeostasis model assessment of insulin resistance (HOMA-IR), atherogenic index ,systolic (SBP) and diastolic blood pressure (DBP).

Results: SCH induced group showed significantly higher mean serum levels of TSH, LCN2, insulin, total cholesterol, low density lipoprotein - cholesterol (LDL-C), triglycerides (TG), CRP than that of control. Also, BMI, calculated HOMA, atherogenic index, SBP and DBP were significantly higher than that of control and all these parameters were positively correlated to LCN2. However, high density lipoproteins-cholesterol (HDL-c) levels were significantly lower than that of control group and negatively correlated with serum LCN2 levels.

Conclusion: Serum lipocalin-2 may be associated and can predict cardiovascular risk factors in SCH.

Key words: Lipocalin-2, subclinical hypothyroidism, cardiovascular disease, atherogenic index, calculated HOMA-IR, rats.

INTRODUCTION

Subclinical hypothyroidism (SCH) is precursor to overt hypothyroidism in which serum TSH levels is elevated while T3 and T4 levels are normal (Cooper, 2001 and Karthick et al., 2013). SCH has been associated with metabolic syndrome, hypertension, dyslipidemia (Cinar et al., 2011). Several studies reported that, SCH has been implicated in development of atherosclerosis (Ochs et al., 2008; Valentina et al., 2011 and Billic-Komarica et al., 2012) and coronary endothelial dysfunction (Biondi et al., 2009 and Gao et al., 2015), leading to an increased e4risk of CVD (Meena et al., 2012).Although other studies have denied that relation (Cappola et al., 2006 and Biondi & Cooper, 2008).
LCN2 cytokine primarily known as a protein of human neutrophils granules (NAGL), was reported to be closely associated with metabolic syndrome, impaired lipid profile, hyper-insulinemia, hyperglycemia, insulin resistance and obesity among humans and mice (Wang et al., 2007).

NGAL up-regulation has been described in several conditions usually associated with hypertension, such as endothelial dysfunction, atherosclerosis and inflammatory vascular damage (Paulsson et al., 2007 and Giaginis et al., 2010). Leoncini et al. (2011) showed that increased NGAL is significantly associated with increase left ventricular mass in primary hypertensive patients. Elevated LCN2 levels were found in patients with coronary heart disease (Choi et al., 2008) and positively correlated with it and with metabolic syndrome in a Chinese cohort (Ni et al., 2013).

Circulating NGAL is a significant predictor of CVD mortality in older adults (Daniels et al., 2012) and in chronic heart failure (van Deursen et al., 2014). Moreover, higher urinary (but not serum) NGAL concentrations are associated with increased cardiovascular mortality risk (Helmersson-Karlqvist et al., 2013). Interestingly, NGAL (serum and urine) levels have been increased in acute (Alvelos et al., 2013) and chronic heart failure patients (Damman et al., 2011). Recently, serum NGAL is associated with cardiovascular fatal and non-fatal events in patients with chronic kidney disease, independently of renal function and inflammation (Solak et al., 2015).

This work aimed to measure serum levels of LCN2 in SCH rat model and its association with some cardiovascular risk factors.

**MATERIAL AND METHODS**

**Animals:** Twenty four adult male albino rats weighting 150-170 g obtained from the animal house of the faculty of veterinary medicine of Zagazig University for use in these experiments. All rats were housed individually in separate steel wire cages (4-6 rats/ cage) at comfortable temperature (20-24°C) and were maintained on a 12h light/dark cycle. Also, were provided free access to food and water and accommodated to animal house conditions for 2 weeks before the experiments took place. The experimental protocol was proved by local medical ethics committee in faculty of medicine of Zagazig University (Institutional Review Board, IRB).

The animals were randomly divided into two equal groups (n=12):

**Group I: Control euothyroid rats:** by administration of physiological saline for 3 months by gavage once daily.

**Group II: sub clinical hypothyroid-induced rats:** induction of SCH was done by administration of (10 mg/kg body weight) methimazole (MMI) by gavage once daily for 3 months (MMI obtained by sigma and dissolved in physiological saline) (Gao et al., 2015).

After 45 days, a rat model of SCH was established, but we continued the methimazole administration and concurrently assessed the TSH, T3 and T4 levels (Gao et al., 2015) to evaluate the long term effects of SCH on cardiovascular risk factors.
At end of 3 months, all animals were weighted and their lengths were measured, BMI was estimated by the equation; body weight (g)/length$^2$ (cm$^2$) = BMI (g/cm$^2$) (Novelli et al., 2007).

**Measurement of Blood Pressure (BP)** according to Zorniak et al. (2010) and Parasuraman & Raveendran (2012); an overnight fasted (8–10 h) each rat was anesthetized with urethane (1200 mg/kg), and placed on a suitable rodent none electrically conductive surgical table. The skin on the ventral side of the neck is shaved and disinfected. The skin was carefully cut open (1.5–2 cm), and a slit incision was made in the platysma muscles. The trachea was identified, small incision was made on the cartilage tissue, and the tracheostomy was performed using a small piece of tracheal intubation tube. One side of the carotid artery was separated from the adjacent connective tissue, and its cephalic end was tied and the cardiac end was clamped with a bulldog clamp and cannulated using a heparinized cannula (0.5 IU/ml in saline). The other end of the cannula connected to a three-way stopcock connected to the pressure transducer and a syringe filled with heparinized saline. Then the carotid artery cannulation site was tied with a thread without obstructing the blood flow in the carotid cannula. Then bulldog clamp was released slowly, ensuring that there was no bleeding at the cannulation site.

After cannulation, the animal is connected to the Power Lab (AD Instruments Pty Ltd, Australia) to record the BP. The pressure cuff of the sphygmanometer was connected to the pressure transducer. Then, the pressure transducer is checked by inflating to a known pressure level. The calibration between the voltage (millivolts) and the pressure (mmHg), and the results are automatically calculated by power Lab software.

**Blood sampling:** Blood samples were taken from the cannula after measuring BP and were allowed to clot for 2 hours at room temperature before centrifuging for 20 minutes at approximately 500 rpm. The separated serum was stored at -20° C.

**Serum analysis:** the following serum levels were measured:

**LCN2 levels** according to Goetz et al. (2002) by using enzyme immunoassay test kit catalog: ELR-LCN2.

**TSH levels** according to Engall (1980) by using rat ELISA kits (Shanghai Sunred Biological Technology Co., Ltd).

**T3** according to Agharanya (1990) by using rat ELISA kits (Shanghai Sunred Biological Technology Co., Ltd).

**T4** according to Schuurs and Van Weeman (1977) by using rat ELISA kits (Shanghai Sunred Biological Technology Co., Ltd).

**TC levels** according to Tietz (1995) by using total cholesterol kits estimation (BioSource Europe S.A).

**HDL-c levels** according to Nauk et al. (1997) by using kits for HDL-cholesterol (BioSource Europe S.A).

**LDL-c** calculated according to Friedewald et al. (1972), LDL=TC-HDL-TG/5.

**TG levels** according to Naito (1989) using triglycerides ESPAS SL kits A (Eltech S.A., Lyon, France).
CRP levels according to Ridker et al. (1998) using CRP Kits (Monobind Inc Lake Forest, Ca 92630, USA).

Insulin levels according to Temple et al. (1992) using INS-EASIA, KAP1251 (BioSource Europe S.A).

Glucose levels according to Tietz (1995) using glucose enzymatic (GOD-PAP)-liquizyme rat Kits (Biotechnology, Egypt).

Calculation of HOMA-IR according to the equation of Sun et al. (2007) modification on Matthews et al. (1985) [HOMA-IR = insulin (µU/mL) x glucose (mg/dL) /405].

Calculation of atherogenic index (AI) according to the equation: Atherogenic index = (total cholesterol-HDL - cholesterol)/HDL-cholesterol (Karthik and Ravikumar, 2011).

Statistical analysis: Data were presented as mean ± SDM. Statistical significance between groups was determined by unpaired t-test; Pearson correlations were performed to determine the correlations between LCN2 and all other parameters. P value of less than 0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS 20 software (SPSS Inc., Chicago, IL, USA).

RESULTS

As in table (1): SCH rats showed significant increase in mean serum TSH and LCN2 levels (p<0.001, p<0.01 respectively) than that of control. However, the groups had no significant differences in terms of T3 and T4 (p>0.05). TSH levels were positively correlated to LCN2 in SCH rats (r=0.72, p<0.01).

BMI, insulin levels and HOMA-IR were significantly elevated in SCH rats when compared with control (p<0.001, p<0.01 and p<0.01 respectively). Moreover, a positive correlation was observed for LCN2 with those parameters [(r=0.69, p<0.05); (r=0.73, p<0.01) and (r=0.708, p<0.05)] respectively, while glucose levels increased but insignificantly (p>0.05) in SCH compared with control and not correlated with LCN2 (r=0.28, p>0.05).

Regarding lipid profiles, CRP levels and AI in SCH rats, there were high significant levels of total cholesterol (p<0.001), LDL-C (p<0.01), TG (p<0.001), CRP (p<0.001) and AI (p<0.001) which were significantly positive correlated to LCN2 (r=0.76; r=0.72; r=0.73; r=0.85, and r=0.72, respectively (p<0.01 in all parameters). However, HDL-cholesterol levels were significantly lower than that of control group (P <0.05) and negatively correlated with serum LCN2 levels (r=0.071, p<0.01).

Concerning blood pressure, as shown in figures (1 and 2) systolic BP and diastolic BP were significantly elevated in SCH group in comparison with control group (P<0.01) and both positively correlated with serum LCN2 levels (r=0.68; r=0.65, P <0.05 respectively).
Figure (1): Blood pressure of rat from control group.

Figure (2): Blood pressure of rat from the SCH group.
Table (1): Serum TSH, T3, T4, LCN2, lipid profile, CRP, glucose, insulin, calculated HOMA-IR, AI, BMI, SBP and DBP in both groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>SCH</th>
<th>P value</th>
<th>r</th>
<th>SCH</th>
<th>P value</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lcn2 (ng/ml)</td>
<td>56.62±10.35</td>
<td>79.9±17.12</td>
<td>&lt;0.01</td>
<td>-0.34</td>
<td>79.9±17.12</td>
<td>&lt;0.01</td>
<td>-0.041</td>
</tr>
<tr>
<td>T3 (ng/mL)</td>
<td>1.45±0.28</td>
<td>1.38±0.42</td>
<td>0.63</td>
<td>-0.007</td>
<td>1.38±0.42</td>
<td>0.63</td>
<td>-0.007</td>
</tr>
<tr>
<td>T4 (ug/dL)</td>
<td>6.41±1.7</td>
<td>5.8 ±1.65</td>
<td>0.38</td>
<td>0.37</td>
<td>5.8 ±1.65</td>
<td>0.38</td>
<td>0.37</td>
</tr>
<tr>
<td>TSH (uIU/mL)</td>
<td>0.0044± 0.0007</td>
<td>0.0081±0.0001</td>
<td>&lt;0.001</td>
<td>0.18</td>
<td>0.0081±0.0001</td>
<td>&lt;0.001</td>
<td>0.18</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>81.2±6.34</td>
<td>84.69±7.76</td>
<td>0.24</td>
<td>-0.29</td>
<td>84.69±7.76</td>
<td>0.24</td>
<td>-0.29</td>
</tr>
<tr>
<td>Insulin (MIU/mL)</td>
<td>16.75±2.34</td>
<td>21.08±3.93</td>
<td>&lt;0.01</td>
<td>-0.07</td>
<td>21.08±3.93</td>
<td>&lt;0.01</td>
<td>-0.07</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.35±0.54</td>
<td>4.4±0.94</td>
<td>&lt;0.01</td>
<td>-0.24</td>
<td>4.4±0.94</td>
<td>&lt;0.01</td>
<td>-0.24</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>86.57±12.6</td>
<td>113.93±16.13</td>
<td>&lt;0.001</td>
<td>0.35</td>
<td>113.93±16.13</td>
<td>&lt;0.001</td>
<td>0.35</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>105.3±5.54</td>
<td>173.78±17.02</td>
<td>&lt;0.001</td>
<td>0.27</td>
<td>173.78±17.02</td>
<td>&lt;0.001</td>
<td>0.27</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>62.9±7.4</td>
<td>79.12±12.6</td>
<td>&lt;0.01</td>
<td>0.017</td>
<td>79.12±12.6</td>
<td>&lt;0.01</td>
<td>0.017</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>51.9±6.5</td>
<td>45.22±7.59</td>
<td>&lt;0.05</td>
<td>0.39</td>
<td>45.22±7.59</td>
<td>&lt;0.05</td>
<td>0.39</td>
</tr>
<tr>
<td>Atherogenic index (AI)</td>
<td>1.05±0.27</td>
<td>2.92±0.65</td>
<td>&lt;0.001</td>
<td>-0.28</td>
<td>2.92±0.65</td>
<td>&lt;0.001</td>
<td>-0.28</td>
</tr>
<tr>
<td>CRP (Ug/ML)</td>
<td>0.87±0.23</td>
<td>2.05±0.49</td>
<td>&lt;0.001</td>
<td>0.034</td>
<td>2.05±0.49</td>
<td>&lt;0.001</td>
<td>0.034</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>92.83±5.52</td>
<td>106.7±12.5</td>
<td>&lt;0.01</td>
<td>-0.26</td>
<td>106.7±12.5</td>
<td>&lt;0.01</td>
<td>-0.26</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>64.5±9.8</td>
<td>79±9.12</td>
<td>&lt;0.01</td>
<td>-0.36</td>
<td>79±9.12</td>
<td>&lt;0.01</td>
<td>-0.36</td>
</tr>
<tr>
<td>BMI (g/cm²)</td>
<td>0.65±0.08</td>
<td>0.81±0.07</td>
<td>&lt;0.001</td>
<td>-0.08</td>
<td>0.81±0.07</td>
<td>&lt;0.001</td>
<td>-0.08</td>
</tr>
</tbody>
</table>

r = correlation with L

DISCUSSION

SCH rat model in current study was confirmed by the significant increase in serum TSH levels, while there was no significant difference in serum levels of both T3 and T4.

Our results had revealed a significant increase in serum LCN2 levels in SCH rats as compared with those of euothyroid rats. Moreover, LCN2 levels were positively correlated with TSH levels in same group.
Our findings of insulin resistance (as measured by HOMA-IR) in SCH rats can be attributed to impaired translocation of GLUT-4 transporters on cell surface of adipose tissue and muscle (Maratou et al., 2009). Similarly, Tuzcu et al. (2005) and Vyakaranam et al. (2014) found insulin resistance and a positive correlation between TSH and insulin in SCH.

Interestingly, LCN2 levels were positively correlated with BMI, insulin levels and HOMA index. This is in accordance with Wang et al. (2007) who suggested that this adipokine might be an independent risk factor for hyperglycemia and insulin resistance in humans. Serum lipocalin-2 levels showed significant positive correlations with BMI in SCH patients (Zorlu et al., 2014).

Concerning lipid profile, our findings came in agreement with Canarís et al. (2000) who showed that total cholesterol levels were higher in subjects with subclinical hypothyroidism. Also, Danese et al. (2000) found that lipid profile was improved with levothyroxine treatment. Furthermore, Owen et al. (2006) and Gao et al. (2015) reported high total cholesterol and LDL-c levels in SCH female patients and SCH rats model, respectively.

Interestingly, even with a mild elevation of serum TSH, SCH is associated with atherogenic lipid profiles in postmenopausal women independent of thyroid hormones (Geng et al., 2015).

Recently, Brenta et al. (2016) showed that women with SCH have higher remnant-like lipoproteins, small dense LDL levels and reduced hepatic lipase activity, which was improved significantly following levothyroxine therapy.

Also, in accordance with our results, Choi et al. (2008) showed that serum LCN2 levels have been positively correlated with serum TG and negatively correlated with serum HDL-c indicating that the related pathogenic mechanism of atherosclerosis may involve disruption of lipid metabolism.

On the other hand, the LCN2 levels were negatively associated with HDL cholesterol (Ni et al., 2013).

Accordingly, we found that atherogenic index (AI) was significantly higher in SCH rats than that of control group, where there was also a positive correlation between serum LCN2 levels and AI in SCH group which was not detected in control group.

SCH represents an independent risk factor for coronary heart disease (Walsh et al., 2005) and may be associated with increases in carotid intima-media thickness and carotid plaque formation (Valentina et al., 2011 and Gao et al., 2013). Moreover, Gao et al. (2015) demonstrated that SCH rats displayed an increased serum endothelin level and a decreased vasodilator nitric oxide level in the SCH rats.

Conversely, Delitala et al., (2015) showed no association between SCH and intima-media thickness or carotid atherosclerotic plaque. Also, Schultz et al. (2011) failed to find any association between SCH and CVD.

Galis and Khatri (2002) elucidated the pathogenic mechanism of LCN2 in atherosclerosis and CAD as it destabilizes the artery plaque and promote atheroscle-
rosis by degrading the vascular basement membrane, leads to increasing endothelial permeability, allowing more white blood cells and inflammatory cytokines to infiltrate the intima. And induced the conversion of macrophages to foam cells (Oberoi et al., 2015).

Interestingly, NGAL is predominantly expressed in atherosclerotic plaques of mice with myocardial infarction (Hemdahl et al., 2006). This indicated that LCN2 could be involved in creating the local and systemic pro-inflammatory environment characteristic for atherosclerosis. Moreover, LCN2 may be used as a prognostic marker to determine the status of CAD progression (Oberoi et al., 2015).

Concerning serum levels of CRP, our results came in contact with results of many reports (Christ-Crain et al., 2003; Guldiken et al., 2008 and Belen, 2015) who showed that CRP levels were elevated in SCH when compared with the control group.

Interestingly, significant correlations between serum levels of LCN2 and serum CRP were showed in patients with metabolic syndrome (Wang et al., 2007) and acute coronary syndrome (Singh et al., 2009 and Ni et al., 2013).

Results regarding blood pressure alterations in SCH are still controversial; in agree with us, Cai et al. (2011) has indicated that SCH is associated with increases in SBP and DBP, moreover a population-based study observed positive linear associations between TSH levels and both systolic and diastolic BP (Asvold et al., 2007). Similarly, SCH is associated with higher systolic BP (Belen, 2015 and Ye et al., 2014), subclinical hypothyroidism and high normal TSH are independent risk factors for hypertension in middle-aged and elderly Chinese women (Jian et al., 2013).

However, a number of studies showed only increased diastolic blood pressure in SCH patients (Saltiki et al., 2008; Anand et al., 2012 and Pesic et al., 2015), others showed no association between SCH and increased BP (Walsh et al., 2006; Iqbal et al., 2007; Duan et al., 2009 and Amouzegar et al., 2016).

In contrast to our results, Gao et al. (2015) showed decreased SBP and DBP in SCH rats. Or decreased only systolic BP (Chen et al., 2013).

Correlation of LCN2 with systolic and diastolic BP in our study was also confirmed by Sachan et al (2014) in patients of hypertensive disorders of pregnancy and in children with nephrotic syndrome (Gheissari et al., 2013). In addition, Aksan et al. (2015) suggested that circulating NGAL concentration might be a useful marker in identifying hypertensive patients with higher risk for cardiovascular mortality. Also, predicts major adverse cardiovascular events after cardiac care unit discharge (Ito et al., 2016).

Furthermore, NGAL level was positively correlated with severity of coronary artery disease in patients with non-ST elevation acute coronary syndrome (Soylu et al., 2015).

On the other hand, our results differ from those of Zorlu et al. (2014) who showed no significant difference in lipocalin-2 levels in patients with subclinical hypothyroidism.
We attributed these controversial findings to spices difference and to heterogeneity of study groups as etiology of hypothyroidism in this study was due to drug induced, but others varied from autoimmune thyroiditis, postpartum thyroiditis to thyroid function insufficiency after radioiodine treatment or after thyroidectomy.

CONCLUSION

Serum lipocalin-2 may be associated and can predict cardiovascular risk factors in subclinical hypothyroid induced rats.

REFERENCES


15. Chen X, Zhang N, Cai Y and Shi J (2013): Evaluation of left ventricular diastolic function using tissue Doppler echocardiography and conventional doppler echocardiography in patients with subclinical hypothyroidism aged...


SERUM LIPOCALIN-2 PREDICTS CARDIOVASCULAR MORBIDITY... 445


الليبوكلين 2 في مصل الدم يبتنا بمخاطر إعتلال القلب و الأوعية الدموية في نموذج الجرذان المصاب بقصور تحت السريري في الغدة الدرقية

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خلفية البحث: قصور تحت السريري في الغدة الدرقية و علاقته ب nguyênات القلب والأوعية الدموية لا تزال مشتركة جدا، و كشفت دراسات سابقة أن مستويات الليبوكلين 2 في الدم مرتبطة بزيادة خطر وفيات القلب والأوعية الدموية.

الهدف من البحث: قياس مستويات الليبوكلين 2 في مصل دم الجرذان المحدث لها قصور تحت السريري في الغدة الدرقية وبحث مدى تأثيره على بعض عوامل الخطورة على القلب والأوعية الدموية.

مواد وطرق البحث: تم استخدام 24 من ذكور الجرذان البضائع البالغة وزنها حوالي 150-170 جرام. وقسمتهم إلى مجموعتين متساويتين: مجموعة جرذان ضابطة و مجموعه جرذان محدث لها قصور تحت السريري في الغدة الدرقية - وذلك بواسطة إعطائها ميثامازول (ملغم / كغم من وزن الجسم) من خلال أنبوب تغذية مرة واحدة يوميا لمدة 3 أشهر. و قد تم قياس كل من هرمونات الغدة الدرقية و الهرمون المحفز لها، وكذلك مستويات الليبوكلين وسكر الدم و الإسولون، و كل أنواع دهون الدم، وبروتين سي المتعامد، ومؤشر كتلة الجسم، ومعادلة مقاومة الإنسولين، و مؤشر تصلب الشرايين، وضغط الدم الإئباضي والإنباضي في كل من المجموعتين.

النتائج: وجد أن في مجموعة الجرذان المحدث لها قصور تحت السريري في الغدة الدرقية مستويات كل من الهرمون المحفز للغدة الدرقية، و الليبوكلين، و الإسولون، و الكولسترول الكلي، و البروتين المنخفض الكثافة، و اللدود الثلاثية، و بروتين سي المتعامد في الدم عالية و ذات دلالة إحصائية عن مثيلاتها في مجموعة الجرذان الضابطة. أيضا، كان مؤشر كتلة الجسم، ومعادلة مقاومة الإنسولين، و مؤشر تصلب الشرايين و ضغط الدم الإئباضي، والإنباضي في نفس ذات دلالة إحصائية أعلى، وذات دلالة إحصائية عنه في مجموعة الجرذان الضابطة، ورتبطت كل هذه المعايير بشكل إيجابي ذو دلالة إحصائية مع الليبوكلين.

وعلى العكس، كانت مستويات البروتين الدهنى العالي الكثافة في مجموعة الجرذان المحدث لها قصور تحت السريري في الغدة الدرقية قليلة، و ذات دلالة إحصائية عن مثيلاتها في المجموعة الضابطة، ووجد أن لها ارتباط سلبي بدلالة إحصائية مع مستويات الليبوكلين.

الاستنتاج: مستويات الليبوكلين 2 في مصل الفئران المحدث لها قصور تحت السريري في الغدة الدرقية عالية و لها ارتباط و تنبأ بمخاطر إعتلال القلب والأوعية الدموية.